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SUBCHRONIC TOXICITY OF VOMITOXIN IN SPRAGUE–DAWLEY RATS

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Abstract—Purified vomitoxin was incorporated into the diet at a level of 20 ppm and fed to male Sprague–Dawley rats *ad lib.* for 90 days. Few clinical signs of toxicity were observed. Rats in the vomitoxin treatment group were less efficient in converting feed into body mass, but there was no feed refusal. Terminal body weight was reduced in the vomitoxin treatment group. There were no statistically significant effects on serum enzyme levels, haematological parameters or tissue lesions, or on liver detoxification systems, as reflected in levels of microsomal cytochrome *P*-450 or in glutathione *S*-transferase activity.

INTRODUCTION

Trichothecene mycotoxins are suspected of causing severe human and animal diseases, such as alimentary toxic aleukia and stachybotriotoxicosis. Vomitoxin (3,7,15-trihydroxy-12,13-epoxytrichothec-9-ene-8-one; deoxynivalenol) is a trichothecene mycotoxin produced primarily by the fungus *Fusarium graminearum* Schwabe (perfect stage *Gibberella zeae*) (Schw.) Petch. *F. graminearum* causes a scab in corn that usually occurs in temperate climates in years that are particularly cool and wet at the time of harvest (Cote, Reynolds, Vesonder *et al.* 1984). In a survey of 1981 corn in Illinois, levels of vomitoxin ranged from 0.1 to 41.6 ppm with a mean of 3.1 ppm (Cote *et al.* 1984). Extensive scab or head blight occurred in Canadian and US wheat in 1980, 1981 and 1982. This resulted in detectable vomitoxin contamination in each of these years. In many of the samples that contained vomitoxin, zearalenone was also present at equal or greater concentrations (Cote *et al.* 1984; Hagler, Tyczkowska & Hamilton, 1984; Jemmali, Ueno, Ishii *et al.* 1978; Mirocha, Schauerhamer, Christensen & Kommedahl, 1979; Neish & Cohen, 1981).

Although LD_{50} values (70 mg/kg, ip, mouse) indicate that vomitoxin is less acutely toxic than other trichothecenes such as T-2 toxin, nivalenol and diacetoxyscirpenol (Ueno, 1977), vomitoxin causes feed refusal, emesis and other effects in swine (Cote *et al.* 1984), indicating that it should be thoroughly investigated. Teratological and reproductive studies on rodents given vomitoxin showed, in rats, effects on weight-gain patterns (Morrissey, 1984; Morrissey & Vesonder, 1985) and, in mice, increased incidences of resorptions and skeletal malformations and an apparent decrease in post-natal survival of pups (Khera, Arnold, Whalen *et al.* 1984; Khera, Whalen, Angers *et al.* 1982). There is a need for longer-term studies to determine the subchronic effects of vomitoxin.

The purpose of this investigation was to determine the effects of a dietary level of 20 ppm purified vomitoxin fed for 90 days on the feed consumption, histology, haematology, and serum enzymes and substrates of male Sprague–Dawley rats. The effects of vomitoxin on microsomal enzyme-metabolizing systems that are known to be involved in the detoxification of other mycotoxins, the cytochrome *P*-450 and glutathione *S*-transferase systems, were also studied.

EXPERIMENTAL

Toxin. Vomitoxin was produced with *Fusarium graminearum* (NRRL 5883) cultured on corn, as previously described (Vesonder, Ellis, Kwolek & DeMarini, 1982). The purity of vomitoxin was estimated to be 96% as determined by gas chromatography and gas chromatography–mass spectrometry. The remaining 4% was 3,15-dihydroxy-12,13-epoxytrichothec-9-ene-8-one. The toxin was incorporated into rodent feed (Charles River Certified Rat Feed, Agway Inc., Syracuse, NY) as previously described (Morrissey, 1984) and the mixture was freeze-dried, broken into cubes, and stored frozen until used (Morrissey & Norred, 1984). Vomitoxin was shown to be stable in chicken feed at room temperature (El-Banna, Hamilton, Scott & Trenholm, 1985).

Experimental design. Male Sprague–Dawley rats (Harlan Sprague–Dawley, Indianapolis, IN), weighing 190–210 g, were acclimatized to the animal facility for 1 wk and then randomly assigned to treatment groups (ten rats/group). Rats were identified by ear notch and housed individually in suspended wire

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cages in racks equipped with automatic watering systems and automatic flushing. The temperature was maintained at 23 ± 3°C and the relative humidity at 50–70%. Fluorescent lighting was turned on at 06.00 hr and off at 18.00 hr to provide a 12-hr light/dark cycle. A control treatment group was given feed treated with solvent (ethanol) prior to mixing and freeze drying, a second group was fed a diet containing 20 ppm (w/w) vomitoxin, and a third group was fed control feed but was pair-fed to the group given vomitoxin. Feed was available *ad lib.* to the first two groups for 90 days, except on the night prior to termination of the experiment, when feed was withheld from all groups. Pair-fed control rats were fed the average amount of control diet consumed by rats in the vomitoxin-treated pair group during the preceding 24 hr. Any feed remaining from pair-fed rats was discarded before new feed was added. Water was freely available to all groups. Feed consumption was recorded daily except between days 60 and 70, when the males were used for breeding in a vomitoxin reproduction experiment (Morrissey & Vesonder, 1985). The rats were weighed weekly and observed twice daily for any unusual signs.

Histopathology. The rats were killed with carbon dioxide on the morning following day 90 of treatment. Tissues (brain, pituitary, sciatic nerve, biceps femoris, salivary glands, cervical lymph nodes, trachea, oesophagus, thyroid, bone marrow, heart, liver, spleen, kidneys, adrenals, stomach, duodenum, pancreas, jejunum, ileum, colon, rectum, urinary bladder, penis, accessory sex glands, testes and epididymides) were preserved in 10% buffered formalin. Routinely prepared paraffin sections were stained with haematoxylin and eosin and examined by light microscopy.

Haematology. Blood (2–3 ml) was collected by cardiac puncture from lightly (ether) anaesthetized rats immediately prior to killing. Serum was separated from clotted blood by centrifuging at 500 **g**. The activities of alkaline phosphatase, creatinine phosphokinase, aspartate aminotransferase and alanine aminotransferase and the concentrations of glucose, triglycerides, total protein and blood urea nitrogen were determined in the serum with an IL Multistat Centrifugal Analyzer (Lexington, MA) using reagent kits. Blood (0.3–0.5 ml) was also collected from the same rats into syringes rinsed with EDTA solution (Sequester-Sol, Fort Lauderdale, FL) to prevent clotting. White-cell and red-cell counts were determined in these samples with a Coulter ZBI counter and haemoglobin content was measured with a Coulter haemoglobinometer. Haematocrit and differential white-cell counts were determined by conventional means.

Hepatic glutathione transferase and cytochrome P-450. Glutathione *S*-transferase activity of liver cytosol was determined with 1-chloro-2,4-nitrobenzene, 1,2-dichloro-4-nitrobenzene and 1,2-epoxy-3-(*p*-nitrophenoxy)propane as substrates by the procedure described by Habig, Pabst & Jakoby (1974). The cytochrome *P*-450 content of 105,000-**g** liver microsomal fractions was assayed by the method of Omura & Sato (1964a, b). Protein concentration was determined by the method of Lowry, Rosebrough, Farr & Randall (1951).

Statistical analyses. Continuous variable data were analysed for statistical differences ($P < 0.05$) by Bartlett's test for homogeneity of variance (Sokal & Rohlf, 1969) and then either by analysis of variance followed by Duncan's multiple range test or by an *F* test followed by the appropriate *t* test (Gad & Weil, 1982). Non-parametric data were analysed by the Kruskall–Wallis test. The distribution free multiple comparison test was used for determining mean separation where there was significant variation among sample means (Gad & Weil, 1982). Chi square with Yates' correction was used to analyse categorical data.

RESULTS

There was no feed refusal or unusual behaviour in any of the animals. Rats given vomitoxin-containing feed consumed significantly more feed than rats in control groups (Table 1). Although initial body weights were similar, after 90 days the rats treated with vomitoxin tended to weigh less than those in the control groups. This suggests that their conversion of feed into body mass was less efficient than that of rats in the control groups.

There were no tissue alterations attributable to vomitoxin treatment in any organ examined. Germinal centres of lymphoid tissue in the spleen and lymph nodes had lesions indicative of non-specific reactive lymphoid hyperplasia in all three groups.

There were few discernible effects of treatment on haematological parameters and differential white-blood-cell counts (Table 2). In the vomitoxin treatment group there were slightly fewer red and white cells, less haemoglobin and more segmented neutrophils than in control groups. None of these changes was statistically significant. Similarly, there were slight but not statistically significant inter-group differences in serum enzyme activities and substrates (Table 3), with the vomitoxin-treated group tending

Table 1. Effect of vomitoxin treatment on feed consumption and body weights of male Sprague–Dawley rats

Treatment group*	Feed consumption (g/day)	Initial body weight (g)	Body weight on day 90 (g)
Control	24.6 ± 2.0[a]	202 ± 6	359 ± 34
Pair-fed control	24.9 ± 1.2[a]	200 ± 4	359 ± 21
Vomitoxin (20 ppm in feed)	27.3 ± 1.9[b]	202 ± 5	347 ± 16

*For further details of treatments see Experimental.
Values are means ± SD for groups of ten rats, and those values with different superscripts differ significantly ($P \leq 0.05$).

Table 2. Effect of 90-day vomitoxin treatment on haematological parameters and white-blood-cell counts in male Sprague–Dawley rats

Parameter	Group*...	Mean values for: Control	Pair-fed control	Vomitoxin (20 ppm in diet)
RBC (10^6/mm^3)		7.1 ± 0.8[a,b]	7.7 ± 1.2[a]	6.4 ± 0.6[b]
WBC (10^3/mm^3)		1.8 ± 1.2	1.8 ± 0.8	1.4 ± 0.5
Haematocrit (%)		38.3 ± 1.7	40.1 ± 3.4	37.8 ± 3.8
Haemoglobin (g/100 ml)		14.0 ± 1.2	15.5 ± 2.9	12.9 ± 1.5
Differential WBC count (% of total)				
Lymphocytes		78.4 ± 8.8	84.1 ± 8.3	79.0 ± 7.7
Segmented neutrophils		12.4 ± 8.3	11.6 ± 8.1	15.6 ± 8.2
Monocytes		2.7 ± 2.1	2.6 ± 2.2	2.3 ± 1.4
Eosinophils		2.8 ± 2.2	1.1 ± 1.3	1.9 ± 1.6
Basophils		1.8 ± 2.5	0.7 ± 1.9	1.2 ± 1.1

RBC = Red blood cells WBC = White blood cells

*For further details of treatments see Experimental.

Values are means ± SD for groups of ten rats and those with different superscripts differ significantly ($P \leq 0.05$).

Table 3. Effect of 90-day vomitoxin treatment on serum enzymes and substrates in male Sprague–Dawley rats

Parameter	Group*...	Mean values for: Control	Pair-fed control	Vomitoxin (20 ppm in diet)
Alkaline phosphatase (U/litre)		92 ± 20	94 ± 20	77 ± 12
Aspartate aminotransferase (U/litre)		78 ± 10	73 ± 9	74 ± 7
Alanine aminotransferase (U/litre)		62 ± 43	50 ± 8	44 ± 9
Creatinine phosphokinase (U/litre)		178 ± 224	87 ± 30	120 ± 64
Total protein (g/100 ml)		6.8 ± 0.4	6.9 ± 0.4	6.8 ± 0.2
Glucose (mg/100 ml)		143 ± 21	155 ± 22	144 ± 21
Blood urea nitrogen (mg/100 ml)		21 ± 3	20 ± 3	19 ± 2
Triglycerides (mg/100 ml)		47 ± 19	46 ± 10	40 ± 8

*For further details of treatments see Experimental.

Values are means ± SD for groups of ten rats.

Table 4. Effect of 90-day vomitoxin treatment on liver size, hepatic protein content and hepatic microsomal *P*-450 in male Sprague–Dawley rats

Treatment group*	Liver:body weight ratio (× 100)	Hepatic microsomal protein (mg/g liver)	Hepatic microsomal cytochrome *P*-450 (nmol/mg protein)	Hepatic soluble protein (mg/g liver)
Control	2.96 ± 0.29	21.4 ± 2.56	0.796 ± 0.091[a]	155.5 ± 14.8
Pair-fed control	2.86 ± 0.14	20.1 ± 1.93	0.885 ± 0.081[b]	148.4 ± 11.3
Vomitoxin (20 ppm in diet)	2.88 ± 0.11	21.3 ± 1.34	0.817 ± 0.055[a,b]	152.3 ± 6.7

*For further details of treatments see Experimental.

Values are means ± SD for groups of ten rats, and those with different superscripts differ significantly ($P \leq 0.05$).

to show lower values for alkaline phosphatase, alanine aminotransferase and triglycerides.

Treatment with vomitoxin for 90 days did not alter the liver detoxification system, as reflected in levels of microsomal cytochrome *P*-450 (Table 4), nor did it increase the liver-to-body weight ratio (Table 4). In comparison with the control group, there was also no effect of vomitoxin treatment on the activity of glutathione *S*-transferase towards a variety of substrates (Table 5).

DISCUSSION

In the study reported here, the administration of feed containing 20 ppm vomitoxin produced very limited effects in male rats. This is in contrast to the significant feed refusal and effects on weight gains that were observed when the same ration was fed to female rats during pregnancy and lactation (Morrissey & Vesonder, 1985). Other investigators who have used comparable rations have also observed effects on pregnant animals and their offspring. Mice that were given 2 mg vomitoxin/kg body weight/day, a dose equivalent to that consumed by rats fed 20 ppm vomitoxin in the present study, had decreased weight gains and significant post-natal mortality (Khera *et al.* 1984). Lactating rats fed 20 ppm vomitoxin had pups with decreased weight gain between days 14 and 21 after gestation (Morrissey & Vesonder, 1985), as did mice fed 1.5 or 2.0 mg

Table 5. Effect of vomitoxin treatment on the activity of glutathione *S*-transferase in the cytosol of livers from male Sprague–Dawley rats

Treatment group*	Glutathione *S*-transferase activity† with substrate		
	CDNB	DCNB	ENPP
Control	$769 \pm 105^{a,b}$	29 ± 8	36 ± 7^{c}
Pair-fed control	760 ± 56^{a}	29 ± 11	23 ± 7^{d}
Vomitoxin (20 ppm in diet)	859 ± 117^{b}	37 ± 14	32 ± 9^{c}

CDNB = 1-Chloro-2,4-dinitrobenzene DCNB = 1,2-Dichloro-4-nitrobenzene
ENPP = 1,2-Epoxy-3-(*p*-nitrophenoxy)propane
*For further details of treatments see Experimental.
†nmol substrate transformed/mg protein/min.
Values are means ± SD for groups of ten rats and those with different superscripts differ significantly ($P < 0.05$).

vomitoxin/kg body weight/day during this same period of development (Khera *et al.* 1984). Male Sprague–Dawley rats fed 1.0 mg vomitoxin/kg body weight and female rats fed 0.25, 0.5 or 1.0 mg vomitoxin/kg for 6 wk had reduced weight gains throughout the study (Khera *et al.* 1984). These were attributed to suppressed feed consumption, in contrast to the decreased feed conversion efficiency observed in the present study.

Swine are sensitive to the effects of vomitoxin-contaminated feed, and exhibit both emesis and feed refusal. Rats have been shown to refuse feed at vomitoxin levels approximating to those that affect swine (Vesonder, Ciegler, Burmeister & Jensen, 1979). No pathological lesions attributable to vomitoxin were observed in pigs given up to 43 ppm vomitoxin in the diet for 21 days (Young, McGirr, Valli *et al.* 1983). In one study, feed refusal and decreased weight gains occurred in pigs fed diets containing 0.3 and 0.7 ppm vomitoxin (Trenholm, Cochrane, Cohen *et al.* 1983). Interestingly, the feed refusal associated with corn naturally contaminated with vomitoxin is greater than that for feeds to which equal concentrations of pure compound have been added (Forsyth, Yoshizawa, Morooka & Tuite, 1977). This indicates that an additional factor may be involved in the swine feed-refusal response.

The decreased ability of domestic animals to convert food into body mass causes adverse economic effects, and the cause may go unrecognized in species that do not vomit or refuse feed in response to the presence of vomitoxin. Animals weakened by exposure to vomitoxin may be subject to secondary effects. Most subacute studies of domestic animals have been carried out in swine, but it is important that other species are studied in order to determine whether subtle effects of vomitoxin occur.

The data from this study indicate that vomitoxin caused a decrease in feed conversion efficiency in adult male rats in the absence of feed refusal, but did not cause overt tissue damage as indicated by a variety of assays. Since exposure to more than one mycotoxin can be expected under field conditions, it is possible that synergistic or additive effects may exacerbate effects caused by a single toxin. Although vomitoxin often occurs with zearalenone, the effects of combined exposure on animals have received only limited study (Marasas, Kriek, van Rensburg *et al.* 1977). Small amounts of zearalenone and vomitoxin in combination could cause abnormalities that result in currently unrecognized effects on animals. Attention should be directed to the interaction of toxins that are commonly encountered under field conditions and are associated with adverse effects in economically important animals.

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